

Genetically heterogeneous and prevalent caliciviruses detected in Canadian swine.

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Abstract

Members of the family *Caliciviridae* (CV) are important pathogens of humans and animals. The genera *Sapovirus* (SaV) and *Norovirus* (NoV) are the most important etiologic agents of food borne gastroenteritis in humans. Both genera have also been found in farm animals in a number of countries which has raised concern about the possibility of zoonotic transmission of these infectious agents. Swine in particular harbors CV strains genetically close to human strains. To investigate their prevalence, diversity and genetic relatedness to human strains, over 200 fecal samples from Canadian swine between 2005 and 2007 were screened for CV by RT-PCR using generic primers. Genetically diverse CVs were detected on the majority of farms: typical swine NoVs were detected exclusively in finisher pigs and heterogeneous SaVs were detected in all age groups. Interestingly, a group of CV strains distantly related to known CV genera were detected and fully characterized. Extensive genomic and phylogenetic analyses suggested that these strains represent a novel genus of CV. Overall our study unveiled previously unknown CV strains and suggest a role for swine in the evolution of CV and as a potential reservoir for human strains.

Introduction

The family *Caliciviridae* is composed of small, non enveloped viruses with positive sense RNA genomes. The family comprises 4 official genera: *Norovirus*, *Sapovirus*, *Lagovirus* and *Vesivirus* and a number of unassigned strains. The genera *Norovirus* (NoV) and *Sapovirus* (SaV) are important human pathogens that cause acute gastroenteritis and represent the leading cause of non-bacterial gastroenteritis in the developed world. Both genera have also been detected in farm animals, notably swine where strains carried by that species have shown close genetic relatedness to human epidemic strains. This has raised questions about the zoonotic potential of these viruses. To gain more insight about the presence and diversity of swine caliciviruses in Canada, over 200 swine fecal samples were screened by RT-PCR using generic calicivirus primers. NoV, SaV and atypical CVs were detected on the majority of farms investigated. Selected strains within each group were characterized further to assess their taxonomy and relatedness to human and known swine strains. Results from these analyses suggest that in addition to typical CVs, Canadian swine harbor novel and previously unknown SaV lineages in addition to strains belonging to a new CV genus.

Materials and methods

A total of 266 fecal samples from 20 farms and 2 abattoirs were collected between 2005 and 2007 from Canadian farms. Viral RNA was extracted from 20 % fecal suspensions using the viral RNA mini kit from Qiagen. One step RT-PCR was performed using CV primers based on conserved motifs situated in the polymerase gene. Amplicons were sequenced and the sequences were aligned and used in phylogenetic analyses using MEGA 3.1 software. Partial and complete genomic sequences of selected strains was performed by 3' and 5' RACE PCR using primers designed based on previously characterized polymerase sequences.

Results and discussion

Swine NoVs were detected exclusively in finisher pigs and phylogenetic analyses grouped all isolates with typical swine strains (figure 1). Our findings support the idea that swine NoV strains detected so far in

different countries seem to have adapted to their host species for some time and might be restricted to them.

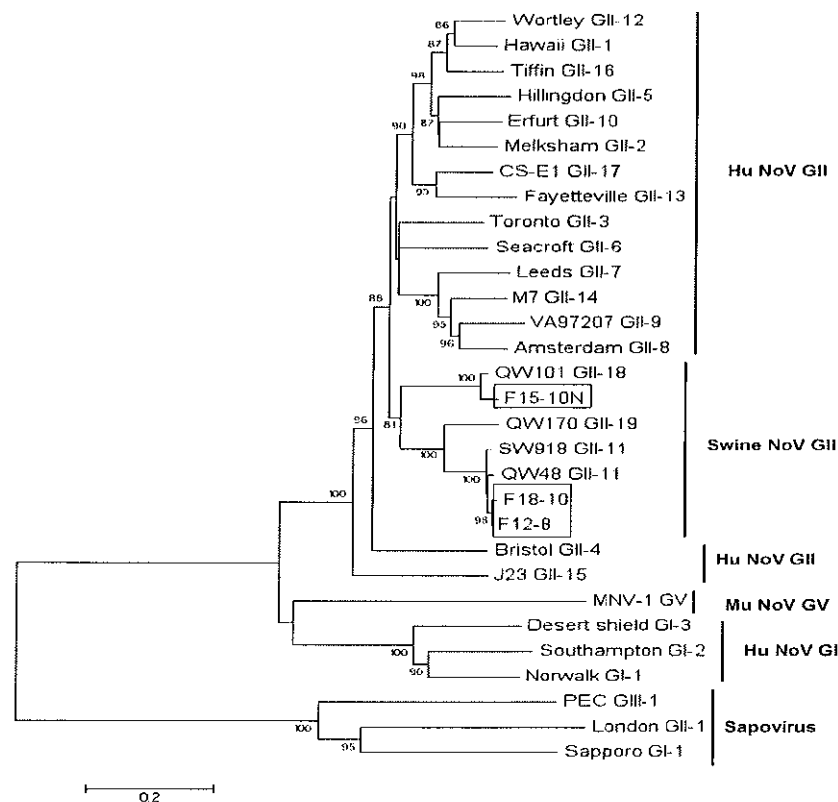
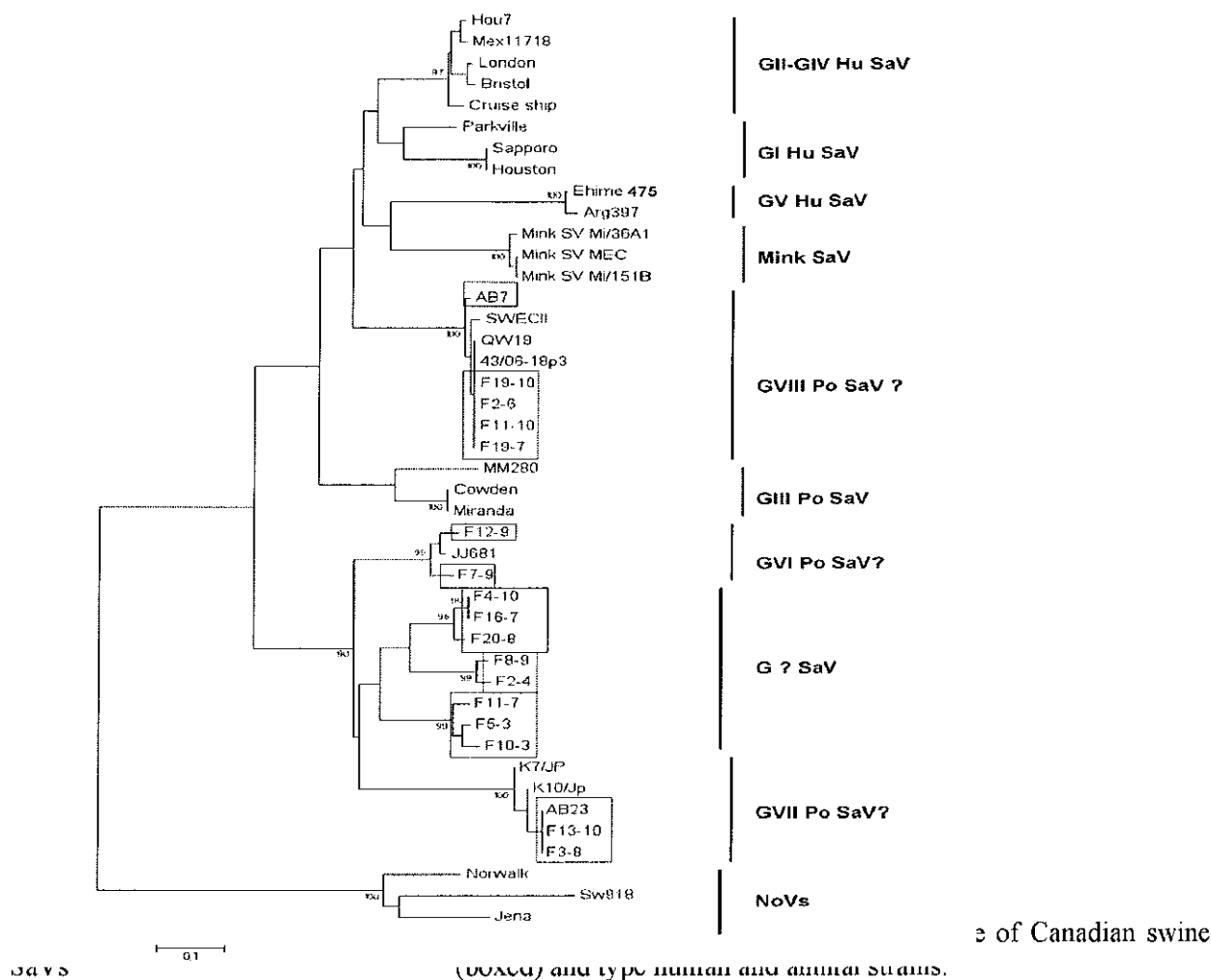


Figure 1. NJ phylogenetic tree of representative NoV strains with type strains from human and swine origin. Canadian swine strains characterized in this study are boxed.

Swine SaVs were detected from pigs of all age groups, were highly prevalent and revealed extensive genetic diversity including a number of strains representing potential novel groups that were previously unknown (figure 2). Our study revealed that this genus is very diverse in Canadian swine and might represent a bigger zoonotic threat than NoVs.

Interestingly, a number of swine CV strains formed a phylogenetic lineage that failed to cluster with any of the contemporary genera. The complete genomic sequence of 3 of these atypical strains was therefore determined. Genomic analyses revealed a gene order reminiscent of CV genomes thereby confirming that these strains are members of the CV family (figure 3). However, phylogenetic and distance analyses suggested that these strains are only distantly related to other CVs. Hence, these atypical CVs appear as members of a new genus of CV previously unknown that we proposed to name *Valovirus* (figure 4).



Conclusion:

Overall, our study revealed unexpected high prevalence and high genetic diversity among Canadian swine caliciviruses. Swine NoVs detected on a number of farms were not highly heterogeneous and appeared closely related to type swine strains. On the other hand, SaV were not only the most prevalent CV genus among pigs of all ages but also showed great genetic diversity that could potentially pose a zoonotic threat. We identified potentially novel SaV strains and complete genomic sequencing and characterization is presently ongoing to attempt identification of taxonomic criteria for this group. Furthermore, the discovery of strains that potentially represent a new genus of *Caliciviridae*, underscores the extensive genetic variability of this family of viruses and the potential reservoir that swine might represent for their evolution.

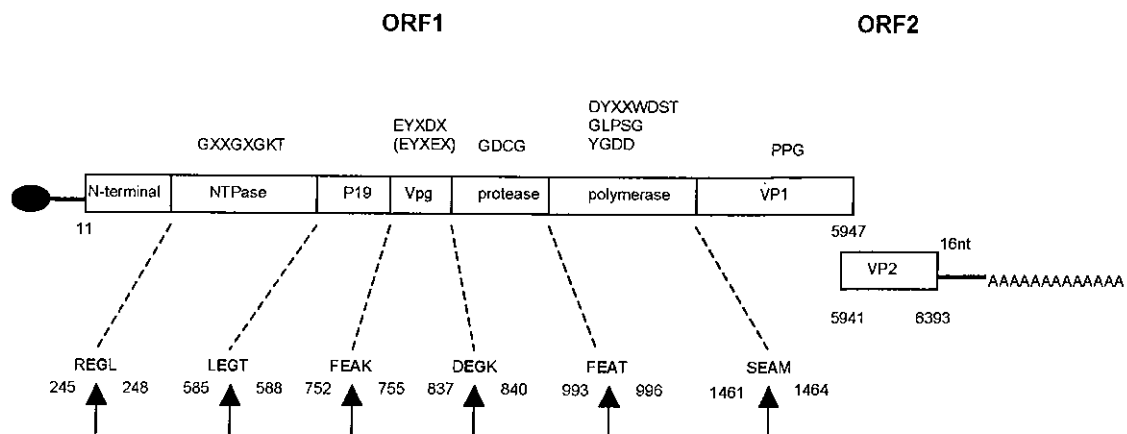


Figure 3. Genomic organization of proposed novel *Valovirus* genus. Conserved aa motifs are shown above each gene and putative cleavage sites are also indicated.

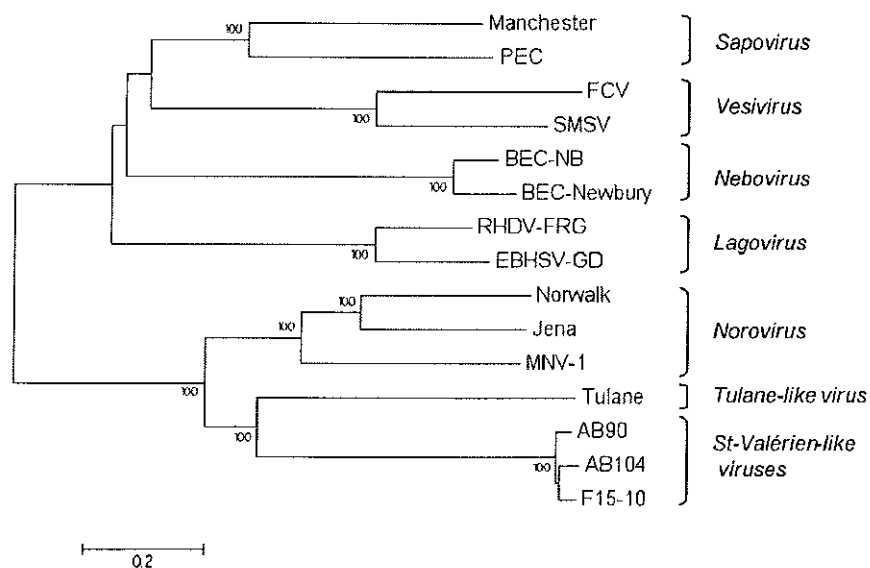


Figure 4. NJ phylogenetic tree based on complete genomic sequence of St-Valérien like viruses and type CV strains of other known genera.